Structures of New and Known Cyanoglucosides from a North American Plant, *Purshia tridentata* DC.¹⁾

Tsutomu NAKANISHI,^{*,a} Masatoshi NISHI,^a Midori SOMEKAWA,^a Hiroko MURATA,^a
Mizuo MIZUNO,^b Munekazu IINUMA,^b Toshiyuki TANAKA,^b Jin MURATA,^c
Frank A. LANG,^d and Akira INADA^a

Faculty of Pharmaceutical Sciences, Setsunan University,^a 45-1 Nagaotoge-cho, Hirakata, Osaka 573-01, Japan, Gifu Pharmaceutical University,^b 5-6-1 Mitahorahigashi, Gifu 502, Japan, Botanical Gardens, Nikko, Faculty of Science, University of Tokyo,^c 1842 Hanaishi-cho, Nikko, Tochigi 321-14, Japan, and Department of Biology, Southern Oregon State College,^d 1250 Siskiyou Boulevard, Ashland, Oregon 97520-5071, U.S.A.

Received May 16, 1994; accepted June 18, 1994

A new cyanoglucoside, purshianin (**1**), was isolated together with a known cyanoglucoside, menisdaurin (**2a**), from stems of *Purshia tridentata* DC. (Rosaceae) collected in Oregon, U.S.A. and the structure was established as (4*S*,6*S*)-(Z)-6-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclohexene-Δ^{1,α}-acetonitrile (**1**) based on chemical and spectral evidence. Notably, the absolute structure of **1** and the revised stereostructure (**2**), (4*S*,6*R*)-(Z)-6-(β-D-glucopyranosyloxy)-4-hydroxy-2-cyclohexene-Δ^{1,α}-acetonitrile for menisdaurin were decided based on combined studies of difference NOE spectra and Dreiding model inspection.

Keywords *Purshia tridentata*; cyanoglucoside; purshianin; menisdaurin; Rosaceae; difference nuclear Overhauser effect spectrum

During our studies on antiviral and antitumor components from Western North American Plants, the ethanol extract from stems of *Purshia tridentata* DC. (Rosaceae), collected in Oregon, U.S.A., was found to show potent inhibitory activity on the human immunodeficiency virus (HIV-1) reverse transcriptase in a screening test.²⁾ In the course of separation of the active component from the extract, a new cyanoglucoside named purshianin (**1**) and a known one, menisdaurin,³⁾ were isolated as major constituents of the extract. Unfortunately, these cyanoglucosides lacked the inhibitory activity, but the structure of purshianin was established to be as shown in formula **1**. In addition, the stereostructure for menisdaurin was revised from **2a**³⁾ to **2**.

The *n*-butanol and aqueous fractions obtained from the

ethanol extract were each subjected to precise separation by column and high-performance liquid chromatography (HPLC) to isolate purshianin (**1**) C₁₄H₁₉NO₇ (Calcd; 313.11616, Found; 313.11706 by high-resolution mass spectrometry), colorless plates of mp 167–169.5 °C (from ethanol), [α]_D²⁰ –90.2° (*c* = 0.45, methanol) and menisdaurin³⁾ in a pure form.

Purshianin (**1**) showed a typical strong nitrile absorption (CN) at 2220 cm^{–1} together with a C=C stretching vibration at 1620 cm^{–1} in the infrared (IR) spectrum and also gave an absorption maximum at 271 nm (log. ε 3.99) in the ultraviolet (UV) spectrum, suggestive of the presence of an αβγδ-unsaturated nitrile group in the molecule.³⁾ The positive ion fast atom bombardment mass spectrum (FAB-MS) afforded the [M+H]⁺ peak at *m/z* 314 and

TABLE I. ¹H-NMR Data (600 MHz, in MeOH-*d*₄) for Purshianin (**1**) and Its Pentaacetate^{a)}

	Purshianin (1)	Pentaacetate of 1
Aglycone		
α-H	5.50, dd, <i>J</i> _{α,3} = 0.8, <i>J</i> _{α,2} = 0.5	5.58, dd, <i>J</i> _{α,3} = 1.0, <i>J</i> _{α,2} = 0.8
2-H	6.25, dddd, <i>J</i> _{2,3} = 10.0, <i>J</i> _{2,4} = 1.8, <i>J</i> _{2,6} = 1.0, <i>J</i> _{2,α} = 0.5	6.34, dddd, <i>J</i> _{2,3} = 10.2, <i>J</i> _{2,4} = 2.2, <i>J</i> _{2,6} = 1.3, ^{b)} <i>J</i> _{2,α} = 0.8
3-H	6.22, dddd, <i>J</i> _{3,2} = 10.0, <i>J</i> _{3,4} = 1.8, <i>J</i> _{3,5eq} = 1.5 ^{b)} <i>J</i> _{3,α} = 0.8	6.11, dddd, <i>J</i> _{3,2} = 10.2, <i>J</i> _{3,4} = 1.8, <i>J</i> _{3,5eq} = 1.8, ^{b)} <i>J</i> _{3,α} = 1.0
4ax(α)-H	4.66, dddd, <i>J</i> _{4,5ax} = 9.5, <i>J</i> _{4,5eq} = 4.5, <i>J</i> _{4,2} = 1.8, <i>J</i> _{4,3} = 1.8	5.54, dddd, <i>J</i> _{4,5ax} = 9.8, <i>J</i> _{4,5eq} = 5.4, <i>J</i> _{4,2} = 2.2, <i>J</i> _{4,3} = 1.8
5eq(α)-H	2.57, dddd, <i>J</i> _{5,5} = 13.5, <i>J</i> _{5,4} = 4.5, <i>J</i> _{5,6} = 4.5, <i>J</i> _{5,3} = 1.5 ^{b)}	2.56, dddd, <i>J</i> _{5,5} = 13.2, <i>J</i> _{5,4} = 5.4, <i>J</i> _{5,6} = 3.8, <i>J</i> _{5,3} = 1.8 ^{b)}
5ax(β)-H	1.60, ddd, <i>J</i> _{5,5} = 13.5, <i>J</i> _{5,4} = 9.5, <i>J</i> _{5,6} = 2.5	1.73, ddd, <i>J</i> _{5,5} = 13.2, <i>J</i> _{5,4} = 9.8, <i>J</i> _{5,6} = 2.5
6eq(β)-H	4.93, ^{c)} dd, <i>J</i> _{6,5eq} = 4.5, <i>J</i> _{6,5ax} = 2.5	4.94, ddd, <i>J</i> _{6,5eq} = 3.8, <i>J</i> _{6,5ax} = 2.5, <i>J</i> _{6,2} = 1.3 ^{b)}
Glucose		
1'-H	4.54, d, <i>J</i> _{1',2'} = 8.0	4.96, d, <i>J</i> _{1',2'} = 8.0
2'-H	3.16, dd, <i>J</i> _{2',3'} = 9.0, <i>J</i> _{2',1'} = 8.0	4.87, dd, <i>J</i> _{2',3'} = 9.6, <i>J</i> _{2',1'} = 8.0
3'-H	3.38, dd, <i>J</i> _{3',4'} = <i>J</i> _{3',2'} = 9.0	5.04, dd, <i>J</i> _{3',4'} = 9.8, <i>J</i> _{3',2'} = 9.6
4'-H	3.29, dd, <i>J</i> _{4',3'} = <i>J</i> _{4',5'} = 9.0	5.28, dd, <i>J</i> _{4',3'} = <i>J</i> _{4',5'} = 9.8
5'-H	3.31, ddd, <i>J</i> _{5',4'} = 9.0, <i>J</i> _{5',6'a} = 5.2, <i>J</i> _{5',6'b} = 2.2	3.90, ddd, <i>J</i> _{5',4'} = 9.8, <i>J</i> _{5',6'a} = 3.9, <i>J</i> _{5',6'b} = 2.5
6'a-H ^{d)}	3.68, dd, <i>J</i> _{6',6'} = 12.0, <i>J</i> _{6',5'} = 5.2	4.26, dd, <i>J</i> _{6',6'} = 12.3, <i>J</i> _{6',5'} = 3.9
6'b-H ^{e)}	3.86, dd, <i>J</i> _{6',6'} = 12.0, <i>J</i> _{6',5'} = 2.2	4.23, dd, <i>J</i> _{6',6'} = 12.3, <i>J</i> _{6',5'} = 2.5
Acetyls	—	1.96, 2.00, 2.05, 2.06, 2.08 (3H, each, all s)

a) Chemical shifts are in δ-values from internal tetramethylsilane (TMS) and are followed by multiplicities and *J*-values (in Hz). b) Long-range coupling in a periplanar W arrangement. c) In this signal, W-type long-range coupling (= *J*_{6,2}) was obscure. d) The 6'-proton resonating at higher field in **1**. e) The 6'-proton resonating at lower field in **1**.

TABLE II. ^1H -NMR Data (600 MHz) for Menisdaurin (2) (in $\text{MeOH}-d_4$ and $\text{DMSO}-d_6$)^{a)}

	$\text{MeOH}-d_4$	$\text{DMSO}-d_6$
Aglycone		
α -H	5.50, ddd, $J_{\alpha,6}=1.3$, $J_{\alpha,2}=0.8$, $J_{\alpha,3}=0.3$	5.62, d, $J_{\alpha,6}=1.8$
2-H	6.29, ddd, $J_{2,3}=10.0$, $J_{2,4}=1.5$, $J_{2,\alpha}=0.8$	6.22, dd, $J_{2,3}=10.0$, $J_{2,4}=1.8$
3-H	6.21, ddd, $J_{3,2}=10.0$, $J_{3,4}=3.5$, $J_{3,\alpha}=0.8$	6.14, dd, $J_{3,2}=10.0$, $J_{3,4}=4.7$
4ax(α)-H	4.36, dddd, $J_{4,5\text{ax}}=6.3$, $J_{4,5\text{eq}}=5.5$, $J_{4,3}=3.5$, $J_{4,2}=1.5$	4.27, dddd, $J_{4,5\text{ax}}=8.0$, $J_{4,4\text{-OH}}=6.7$, $J_{4,5\text{eq}}=5.5$, $J_{4,3}=4.7$, $J_{4,2}=1.8$
4eq(β)-OH	—	5.10, d, $J_{4\text{-OH},4}=6.7$
5eq(α)-H	2.25, ddd, $J_{5,5}=13.2$, $J_{5,4}=5.5$, $J_{5,6}=3.5$	2.26, ddd, $J_{5,5}=12.5$, $J_{5,4}=5.5$, $J_{5,6}=4.0$
5ax(β)-H	2.04, ddd, $J_{5,5}=13.2$, $J_{5,6}=8.0$, $J_{5,4}=6.3$	1.67, ddd, $J_{5,5}=12.5$, $J_{5,6}=10.3$, $J_{5,4}=8.0$
6ax(α)-H	4.93, ddd, $J_{6,5\text{ax}}=8.0$, $J_{6,5\text{eq}}=3.5$, $J_{6,\alpha}=1.3$	4.74, ddd, $J_{6,5\text{ax}}=10.3$, $J_{6,5\text{eq}}=4.0$, $J_{6,\alpha}=1.8$
Glucose		
1'-H	4.55, d, $J_{1',2'}=7.5$	4.41, d, $J_{1',2'}=7.5$
2'-H	3.34, dd, $J_{2',3'}=9.0$, $J_{2',1'}=7.5$	3.15, ddd, $J_{2',3'}=8.7$, $J_{2',1'}=7.5$, $J_{2',2'\text{-OH}}=4.2$
2'-OH	—	4.96, d, $J_{2'\text{-OH},2'}=4.2$
3'-H	3.39, dd, $J_{3',2'}=J_{3',4'}=9.0$	3.18, ddd, $J_{3',2'}=J_{3',4'}=8.7$, $J_{3',3'\text{-OH}}=4.5$
3'-OH	—	4.92, d, $J_{3'\text{-OH},3'}=4.5$
4'-H	3.29, dd, $J_{4',3'}=J_{4',5'}=9.0$	3.02, ddd, $J_{4',3'}=J_{4',5'}=8.7$, $J_{4',4'\text{-OH}}=5.4$
4'-OH	—	4.91, d, $J_{4'\text{-OH},4'}=5.4$
5'-H	3.34, ddd, $J_{5',4'}=9.0$, $J_{5',6'\text{a}}=6.2$, $J_{5',6'\text{b}}=2.2$	3.17, ddd, $J_{5',4'}=8.7$, $J_{5',6'\text{a}}=6.2$, $J_{5',6'\text{b}}=2.3$
6'a-H ^{b)}	3.67, dd, $J_{6',6'}=11.8$, $J_{6',5'}=6.2$	3.43, ddd, $J_{6',6'}=11.8$, $J_{6',5'}=6.2$, $J_{6',6'\text{-OH}}=5.2$
6'b-H ^{c)}	3.89, dd, $J_{6',6'}=11.8$, $J_{6',5'}=2.2$	3.69, ddd, $J_{6',6'}=11.8$, $J_{6',6'\text{-OH}}=6.8$, $J_{6',5'}=2.3$
6'-OH	—	4.16, dd, $J_{6'\text{-OH},6'\text{b}}=6.8$, $J_{6'\text{-OH},6'\text{a}}=5.2$

a) Chemical shifts are in δ -values from internal TMS and are followed by multiplicities and J -values (in Hz). b) The 6'-proton resonating at higher field. c) The 6'-proton resonating at lower field.

an intense fragment ion at m/z 152 ($[\text{M}+\text{H}]^+ - 162$ [hexose unit]), indicating that purshianin is a monoglycoside and carries a hexosyl residue as the sugar part. The D-glucosyl moiety in purshianin was identified as the corresponding thiazolidine derivative by gas liquid chromatography (GLC)(see Experimental). Detailed studies of the proton and carbon-13 nuclear magnetic resonance (^1H - and ^{13}C -NMR) spectra of purshianin were performed with the aid of the INEPT (insensitive nuclei enhanced by polarization transfer) method and two dimensional (2D) techniques such as ^1H - ^1H and ^{13}C - ^1H shift-correlated (COSY), nuclear Overhauser effect (NOESY), and heteronuclear multiple bond correlation (HMBC) spectroscopy, and all protons and carbons were assigned as shown in Tables I and III. The assignments (chemical shifts, multiplicities, and coupling constants) for all glucosyl protons (Table I) and carbons (Table III) substantiated the presence of a β -D-glucopyranosyl ($^4\text{C}_1$ form) residue in the purshianin molecule. On the other hand, the assignments established for all protons (Table I) and carbons (Table III) of the aglycone, taking account of the cyanomethylene geometry which was decided from the NOESY cross peak observed between α -H and 2-H, indicated the absolute structure for the aglycone part to be (Z)-6 α (ax),4 β (eq)-dihydroxy-2-cyclohexene- $\Delta^{1,2}$ -acetonitrile (the aglycone part of the formula **1**) or its antipode (the aglycone part of the formula **1a**). Analogously, ^1H - and ^{13}C -NMR assignments for the pentaacetate of purshianin were successfully achieved (Tables I, III) and they supported the above aglycone stereostructure.

As the next step of the structural elucidation, the location of the β -D-glucopyranosyl residue on the aglycone was examined as follows. In the ^1H - and ^{13}C -NMR spectra of purshianin and its pentaacetate (Tables I, III), both 4ax-H and 4-C in the pentaacetate resonated at lower field

TABLE III. ^{13}C -NMR Spectral Data for Purshianin (1), Its Pentaacetate, and Menisdaurin (2) (150 MHz, $\text{MeOH}-d_4$, δ_{C} , ppm from TMS)^{a)}

Carbon	1	Pentaacetate ^{b)} of 1	2
Aglycone			
α -C	98.73 (d)	99.54 (d)	97.61 (d)
1-C	157.09 (s)	154.14 (s)	157.77 (s)
2-C	127.46 (d)	128.31 (d)	128.43 (d)
3-C	144.27 (d)	137.79 (d)	141.27 (d)
4-C	65.28 (d)	68.16 (d)	66.0 (d)
5-C	38.35 (t)	34.73 (t)	36.74 (t)
6-C	75.23 (d)	75.43 (d)	73.22 (d)
CN	119.05 (s)	117.48 (s)	118.74 (s)
Glucose			
1'-C	104.06 (d)	101.65 (d)	102.25 (d)
2'-C	75.77 (d)	72.73 (d)	75.21 (d)
3'-C	79.04 (d)	74.29 (d)	78.72 (d)
4'-H	72.40 (d)	69.77 (d)	72.44 (d)
5'-H	78.84 (d)	73.14 (d)	78.82 (d)
6'-H	63.73 (t)	62.97 (t)	63.84 (t)

a) Assignments and multiplicities (in parentheses) were determined based on ^1H - ^{13}C COSY, INEPT, and HMBC experiments. b) Methyl (all q) and carbonyl (all s) carbon signals due to acetyl groups in the pentaacetate of **1** are as follows; 20.94, 20.74, 20.69, 20.59, 20.57 (Me $\times$ 5), 172.49, 171.95, 171.66, 171.38, 171.29 (CO $\times$ 5).

than those in purshianin, whereas both 6eq-H and 6-C of the pentaacetate resonated at approximately the same field compared with the corresponding proton and carbon signals of purshianin. In addition, there was an intense cross peak between 6eq-H and the glucosyl anomeric proton in the NOESY and HMBC spectra. These NMR studies proved that the β -D-glucopyranosyl moiety is linked at 6ax-OH of the aglycone. Based on the accumulated evidence, purshianin should be assigned to the structure **1** or **1a**.

Finally, the absolute structure for purshianin was

Chart 1

determined as follows. In the difference nuclear Overhauser effect (NOE) experiments on purshianin (in methanol- d_4), strong difference NOE was observed between the glucosyl anomeric proton ($1'\text{-H}$) and the 6eq-H and between the glucosyl $6'\text{-b-H}$ (the lower field proton of the two $6'$ -protons) and the 5eq-H . These ^1H - ^1H NOE relations were confirmed by the presence of corresponding intense cross peaks in the NOESY spectrum. The steric feature suggested by these NOE findings is, based on Dreiding model inspection, consistent with the absolute structure **1** (Fig. 1) but not with structure **1a**. In conclusion, the absolute structure of purshianin is defined as (*Z*)-6 α -(β -D-glucopyranosyloxy)-4 β -hydroxy-2-cyclohexene- $\Delta^{1,2}$ -acetonitrile [= (4*S*, 6*S*)-(*Z*)-6-(β -D-glucopyranosyloxy)-4-hydroxy-2-cyclohexene- $\Delta^{1,2}$ -acetonitrile] (**1**).

The identity of the isolated menisdaurin with an authentic sample was corroborated as follows. The physical and spectral data of the isolated menisdaurin [mp, $[\alpha]_D$, IR (KBr), UV (EtOH), and ^1H -NMR (Table II, MeOH- d_4)] and its pentaacetate [mp, $[\alpha]_D$, IR (KBr), electron impact mass (EI-MS), and ^1H -NMR (400 MHz, CDCl_3)] were in agreement with reported values for menisdaurin and its pentaacetate.³¹ About fifteen years ago, Takahashi *et al.* tentatively proposed the structure (**2a**), (4*R*, 6*S*)-(*Z*)-6-(β -D-glucopyranosyloxy)-4-hydroxy-2-cyclohexene- $\Delta^{1,2}$ -acetonitrile for menisdaurin, based on the relation with an artificial aglycone of menisdaurin, menisdaurilide, the structure (**3a**) of which had been inferred from a circular dichroism (CD) study,³¹ and this structure (**2a**) has so far been adopted as the absolute

structure of menisdaurin.⁴⁾ Recently, Yamasaki *et al.* isolated naturally occurring menisdaurilide together with menisdaurin from *Sinomenium acutum* (Menispermaceae) and revised the absolute structure for menisdaurilide to **3** on the basis of X-ray analytical evidence.⁵⁾ In the same paper, the revised structure (**2**) for menisdaurin was reported but no evidence and explanation for this structural revision was described.

Comparing the structures established for purshianin (**1**) and bauginin (**4**),⁶⁾ we considered that the structure (**2**),

Fig. 1. The Most Probable Three-Dimensional Conformation of Purshianin (**1**) in Methanol-*d*₄, Inferred from the Present NOE Experiments

rather than (**2a**), seemed more plausible for menisdaurin and so we reinvestigated the structure of menisdaurin.

Detailed ¹H- and ¹³C-NMR assignments [Tables II (both in methanol-*d*₄ and dimethyl sulfoxide(DMSO)-*d*₆) and III (in methanol-*d*₄), respectively] of the isolated menisdaurin were performed with the aid of the ¹H-¹H, ¹H-¹³C COSY, and NOESY experiments and the stereostructure (**2**) for menisdaurin was decided based on detailed analyses of the difference NOE spectra measured in DMSO-*d*₆ (Table II) as follows. When the 6ax-proton was irradiated, strong difference NOE was seen at the 4ax-, the glucosyl anomeric, and the glucosyl 6'-hydroxylic protons, together with weak difference NOE at the glucosyl 6'a- and 6'b-protons. In addition, irradiation of the glucosyl anomeric proton gave a strong difference NOE at the 5eq-proton.

These NOE data, based on Dreiding model inspection, are consistent only with the stereostructure **2** (Fig. 2) of the two candidates (**2**, **2a**). This stereostructure (**2**) established for menisdaurin was further corroborated by additional ¹H-NMR evidence as follows. The glucosyl 2'-proton of the isolated menisdaurin (in methanol-*d*₄; Table II) resonated at lower field (δ 3.34), compared with the corresponding 2'-proton (δ 3.16) of purshianin (**1**) (in methanol-*d*₄; Table I). This paramagnetic shift appears to be due to anisotropy of the cyano group in the menisdaurin molecule. Based on Dreiding model inspection, the implied steric relation between the 2'-H and the CN residue is consistent with the stereostructure **2**, but not with **2a**. Thus, menisdaurin should be expressed by the revised structure (**2**), (Z)-6 β -(β -D-glucopyranosyloxy)-4 β -hydroxy-2-cyclohexene- $\Delta^{1,\alpha}$ -acetonitrile [= (4*S*,6*R*)-(Z)-6-(β -D-glucopyranosyloxy)-4-hydroxy-2-cyclohexene- $\Delta^{1,\alpha}$ -acetonitrile].

Isolation and characterization of the HIV-1-inhibitory active constituent from the ethanol extract are still under study.

Fig. 2. The Most Probable Three-Dimensional Conformation of Menisdaurin (**2**) in DMSO-*d*₆, Inferred from the Present NOE Experiments

Experimental

General Remarks All melting points were recorded on a Yanagimoto micro melting point apparatus without correction. The spectral data were obtained on the following instruments: optical rotation on a JASCO DIP-140, IR on a JASCO A-302, UV on a Shimadzu UV-300, ^1H - and ^{13}C -NMR on a GE-OMEGA 600 and a JEOL GX-400, and EI-MS, high-resolution MS, and positive ion FAB-MS on a JEOL JMS-DX 300. GLC was carried out on a Shimadzu GC-7AG under the following conditions: capillary column; TC-1 (0.32 mm i.d. $\times$ 30 m, GL Sciences Inc.; a column similar to SE-30); detector, hydrogen flame ionization detector; column temperature, 225 $^\circ$; injection temperature, 270 $^\circ$; carrier N_2 gas, 1.1 ml/min; split ratio, 1/70. As silica gel for column chromatography, Kieselgel 60 (Merck, 230–400 mesh) was used. Preparative HPLC was performed on a JAI LC-908 with a JAIGEL-GS 310 column (20 mm i.d. $\times$ 50 cm) at an eluant (MeOH) flow rate of 3 ml/min.

Plant Material Aerial parts of wild *Purshia tridentata* were collected in 1991 in Ashland, Oregon, U.S.A. and a voucher specimen has been deposited in the Herbarium of the Botanical Gardens, Faculty of Science, University of Tokyo.

Extraction and Isolation of Purshianin (1) and Menisdaurin (2) The air-dried aerial parts (2.83 kg) were rinsed in acetone and then divided into leaves (including fruits) and stems. The stems (1.78 kg) were extracted twice with EtOH (10 l) at room temperature for four weeks and then the solvent was evaporated off under reduced pressure to give an extract (118 g). A part (100 g) of the extract was suspended in H_2O (600 ml) and the suspension was extracted successively with AcOEt (300 ml $\times$ 3) and *n*-BuOH (300 ml $\times$ 3) to afford AcOEt (55.4 g) and *n*-BuOH (16.0 g) extracts together with the residual aqueous extract (29.0 g). The aqueous extract (18.0 g) was chromatographed on silica gel (1 kg), eluting successively with the lower phase of each of CHCl_3 -MeOH- H_2O (11:3:1), (65:35:10), and a mixture of CHCl_3 -MeOH- H_2O (6:4:1) to get 11 fractions (Nos. 1 to 11). Fraction No. 6 (2.08 g) containing **2** and fraction No. 8 (0.70 g) containing **1** were eluted with the lower phase of CHCl_3 -MeOH- H_2O (65:35:10) in that order, and further purified by preparative HPLC separation and subsequent recrystallization from EtOH to give menisdaurin (**2**) (973 mg) and purshianin (**1**) (200 mg), respectively. An analogous chromatographic and HPLC separation of the *n*-BuOH extract afforded additional amounts of **1** (30 mg) and **2** (259 mg).

Purshianin (1): Colorless plates from EtOH, mp 167–169.5 $^\circ\text{C}$, $[\alpha]_{\text{D}}^{20}$ -90.2° ($c=0.45$, MeOH). IR (KBr) cm^{-1} : 3450, 3350 (OH), 2220 (CN), 1620 (C=C). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 271 nm (3.99). Positive ion FAB-MS m/z [%]: 314 [(M+1) $^+$, 26], 163 [(hexose unit + 1) $^+$, 4], 152 [(M+1) $^+$ - 162 (hexose unit), 39]. ^1H - and ^{13}C -NMR: see Tables I and III. High-resolution MS: 313.11706 (M^+ , Calcd for $\text{C}_{14}\text{H}_{19}\text{NO}_7$ 313.11616).

Menisdaurin (2): Colorless plates from EtOH, mp 169.5–172.0 $^\circ\text{C}$ [lit.³⁾ mp 175–176 $^\circ\text{C}$; lit.⁵⁾ mp 174–175 $^\circ\text{C}$], $[\alpha]_{\text{D}}^{20}$ -214.5° ($c=0.65$, MeOH) [lit.³⁾ $[\alpha]_{\text{D}}^{15}$ -185.4° ($c=1.00$, MeOH); lit.⁵⁾ $[\alpha]_{\text{D}}^{27}$ -212° ($c=1.00$, MeOH)]. IR (KBr), UV (EtOH), and ^1H -NMR (MeOH- d_4 , Table II): Essentially the same as the reported data.³⁾ ^1H -NMR (DMSO- d_6) and ^{13}C -NMR (MeOH- d_4): Given in Tables II and III, respectively (reported here, for the first time).

Acetylation of 1 A solution of **1** (10 mg) in Ac_2O (2 ml) and pyridine (5 ml) was allowed to stand at room temperature for 2 d. The solution was poured into ice-water and extracted with CHCl_3 . The CHCl_3 layer was washed with H_2O , dried over MgSO_4 , and evaporated to dryness. The residue was recrystallized from MeOH to give the corresponding

pentaacetate (6 mg), colorless plates of mp 123.5 $^\circ\text{C}$, $[\alpha]_{\text{D}}^{20}$ -69.2° ($c=0.50$, MeOH). IR (KBr) cm^{-1} : 2220 (CN), 1745 (acetate). UV $\lambda_{\text{max}}^{\text{EtOH}}$ nm (log ϵ): 261 nm (4.14). EI-MS m/z (%): 523 (M^+ , 14), 331 (tetraacetyl glucosyl unit, 50), 169 (100). ^1H - and ^{13}C -NMR: Given in Tables I and III, respectively. High-resolution MS: 523.16838 (M^+ , Calcd for $\text{C}_{24}\text{H}_{29}\text{NO}_{12}$ 523.16888).

Acetylation of 2 A solution of **2** (30 mg) in a mixture of Ac_2O (2 ml) and pyridine (5 ml) was allowed to stand at room temperature for 2 d and the reaction mixture was worked up in the same manner as described for the acetylation of **1**. The product obtained was recrystallized from MeOH to give the pentaacetate of **2** (15 mg), colorless needles of mp 184.5–186.0 $^\circ\text{C}$ [lit.³⁾ mp 178–179 $^\circ\text{C}$], $[\alpha]_{\text{D}}^{20}$ -152.0° ($c=0.5$, MeOH) [lit.³⁾ $[\alpha]_{\text{D}}^{25}$ -160.2° ($c=1.00$, MeOH)]. IR (KBr), UV (EtOH), EI-MS, and ^1H -NMR (400 MHz, CDCl_3): Essentially the same as the published data.³⁾

Determination of D-Configuration of the Glucosyl Moiety in 1 and 2⁷⁾ A solution of **1** (3 mg) in 10% H_2SO_4 -EtOH (1:1; 3 ml) was refluxed for 15 h. The reaction mixture was neutralized with Amberlite IRA-410 resin, and the solvents were evaporated under reduced pressure to afford a residue containing glucose. The residual glucose was subjected to preparation of the thiazolidine derivative, followed by trimethylsilylation, and GLC analysis according to the reported procedure,⁷⁾ and the D-configuration of the glucose was confirmed based on direct comparisons with standard samples of D- and L-glucose (t_R : D-glucose, 23'30"; L-glucose, 24'39"). In the same manner as described for the hydrolysis and analysis of **1**, the configuration of the glucosyl moiety in **2** (3 mg) was examined and determined to be D.

Acknowledgement This study was supported in part by a Grant-in-Aid for Scientific Research (No. 03042017) from the Ministry of Education, Science, and Culture, Japan. Thanks are due to Ms. J. Severs and her colleagues, US Department of Interior, Bureau of Land Management, Medford, Oregon and to Mr. D. Kennedy for help in collection of the plant material.

References and Notes

- 1) A part of this work was presented at the 113th Annual Meeting of the Pharmaceutical Society of Japan, Osaka, March 1993, Abstract Papers, Vol. 2, p. 213.
- 2) T. Nakanishi, A. Inada, H. Murata, M. Iinuma, T. Tanaka, H. Yamamoto, M. Kato, M. Mizuno, H. Nakane, K. Ono, F. A. Lang, J. Murata, *Shoyakugaku Zasshi*, **47**, 295 (1993).
- 3) K. Takahashi, S. Matsuzawa, M. Takani, *Chem. Pharm. Bull.*, **26**, 1677 (1978).
- 4) The following two groups^{4a,b)} have so far isolated menisdaurin and identified it by comparison of physicochemical and spectral data with those of an authentic specimen.³⁾ However, both groups erroneously cited the revised absolute structure (**2**) instead of the absolute structure (**2a**) published for menisdaurin,³⁾ though no evidence or explanation for this structural revision was provided. a) K. Ueda, K. Yasutomi, I. Mori, *Chem. Lett.*, **1983**, 149; b) A. Nahrstedt, V. Wray, *Phytochemistry*, **29**, 3934 (1990).
- 5) H. Otsuka, A. Ito, N. Fujioka, K. Kawamata, R. Kasai, K. Yamasaki, T. Satoh, *Phytochemistry*, **33**, 389 (1993).
- 6) C.-C. Chen, Y.-P. Chen, H.-Y. Hsu, *Lloydia*, **48**, 933 (1985).
- 7) S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.*, **34**, 1843 (1986).